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Biochemical Characterization and Bioactivity of Red Alga, *Ceratodictyon spongiosum* (Zanardini) from Goso-on, Carmen, Agusan Del Norte, Philippines

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A B S T R A C T

Rhodophyta, present in marine and freshwater habitats, are the most varied group of seaweeds and an essential source of biochemical compounds. This study aimed to analyze the bioactive properties and composition of Rhodophyta *Ceratodictyon spongiosum* (Zanardini). The species was subjected to FTIR, antibacterial, cytotoxic, and proximate analyses. The proximate composition of ethanolic extract *C. Spongiosum* included 10.28±0.04% moisture, 41.09±0.09% ash, 36.00±0.38% crude protein, 2.63±0.02% crude fat, 1.21±0.02% crude fiber, and 0.65±0.02% total sugar. The FTIR spectrum subsequently reveals the presence of numerous functional groups, including alcohol, amine salt, carboxylic acid, alkene, amine, alkane, conjugated alkene, ester, primary amine, aromatic group, ether, and oxy. The species also demonstrated highly potent antibacterial activity against *S. aureus* (24.67±0.58) and *E. coli* (22.67±0.58). Moreover, after 6 hours in BSLT, the extracted sample resulted in a 3% mortality rate at just 100 ppm, yielding an LC_{50} of 928.86 ppm. Nevertheless, at 24 hours, the extracts sample produced high percentage mortality of 45.56 percent, 96.6 percent, and 100 percent at 1, 10, and 100 ppm, respectively, and acquired an LC_{50} of 0.96 ppm, which was extremely poisonous to brine shrimp nauplii. Additional research on *Ceratodictyon spongiosum* in Mindanao is recommended because it contains biological components that might lead to the discovery of novel medications and food products for industrial and medicinal uses.

Keywords: *Ceratodictyon, proximate composition, cytotoxicity, antibacterial, functional group*

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1 Introduction

Rhodophyta, also known as red algae, is the most diverse group of seaweed (Ismail et al. 2020). It is multicellular and found in marine and freshwater ecosystems. Found in-depth intertidal zones and a few terrestrial or subaerial representatives (Gurgel et al. 2007). The species is rich in polysaccharides and floridean starch (Khotimchenko et al. 2002). It also contains sulfated galactans such as carrageenans and agars (Ismail et al. 2015). As evidence shows, Rhodophyta macroalgae contain numerous biochemical constituents, mainly lipids, proteins, and carbohydrates (Ismail et al. 2015). This species is a critical source and has been found to contain high amounts of macronutrients, such as pigments, minerals, and vitamins (Sudhakar et al. 2018). Compounds related to antimicrobial, antiviral, anti-inflammatory, and other viral diseases (Pérez et al. 2016), as well as human food production and cosmetics (McHugh 2003).

In the Philippines, active searches for the biological properties of Rhodophyta have become widespread. Goso-on, Carmen, Philippines' marine environment is home to a great diversity of marine organisms (Walag & Rosario 2018; Walag et al. 2019), including *Ceratodictyon spongiosum* (Zanardini). This species, which belongs to the division Rhodophyta, is a filamentous red alga that lives in symbiosis and has a sponge-like appearance. It was first explored in Mahatao, Batanes, Philippines, from 1999 to 2002, with its assemblage. *Ceratodictyon spongiosum* and *Haliclona cymaeformis* exhibited a compound known as p-sulfooxyphenylpyruvic acid (Bugni et al. 2002). However, the current literature on the biochemical components of *C. spongiosum* might potentially have is lacking, despite its wide occurrence in Mindanao (Baleta & Nalleb 2016). Further research is needed to determine the applications of this biological species. This study evaluates the biochemical composition of the Rhodophyta *Ceratodictyon spongiosum* (Zanardini) found in Gosoon, Carmen, Agusan del Norte, Mindanao, Philippines. Additionally, it investigates the bioactivity and cytotoxicity of this species.

2 Materials and Methods

Locale of the Study

Samples were collected during partly cloudy weather on the 27th day of August 2021 in the specific coastal area of Goso-on, Carmen, Agusan del Norte, Philippines (09º 04' 41.3" N and 125º 13' 17.2" E ArcGis- Software) (Figure 1). Water quality parameters were determined in the following areas: pH, temperature (°C), salinity (ppt), conductivity (mS/cm), and Oxidation-reduction potential (mV) using an OAKTON PC 450 waterproof portable meter.

Sample Collection and Extraction

Approximately 1800 g of wet samples were collected in shallow water, placed in a polythene bag, transported, cleaned, sterilized with distilled water, and air-dried for 14 days in a warm, closed environment. The red seaweed *Ceratodictyon spongiosum* has been documented. They were categorized at the species level using the Seaweeds Guide Publication, an online resource that facilitates species identification. Voucher specimens were prepared for the herbarium.

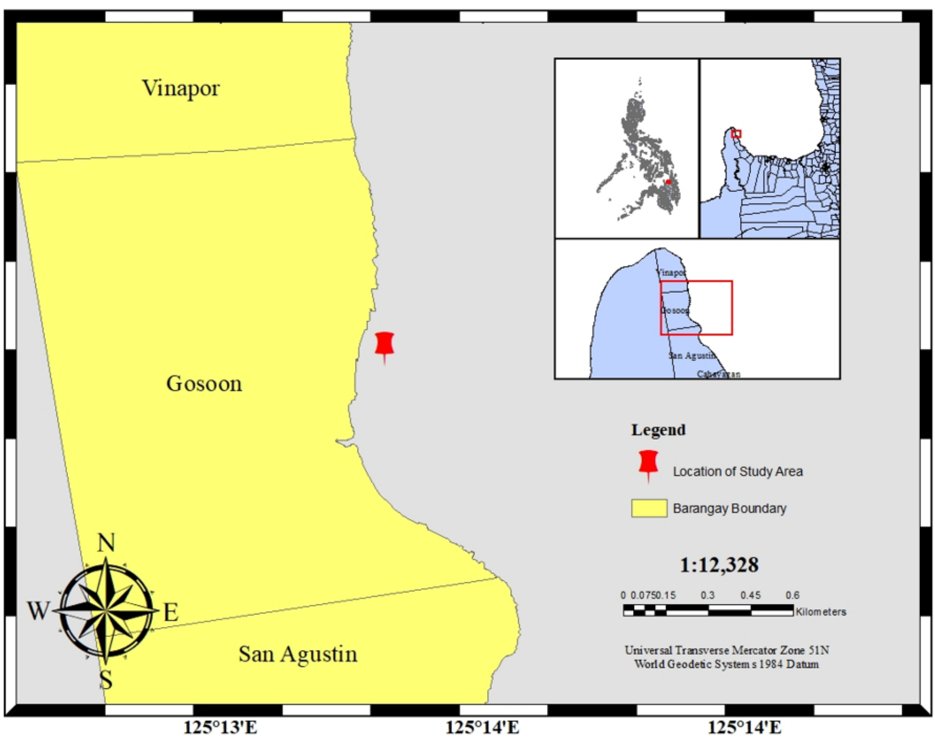

Figure 1. Map showing the location of the sampling area at Gosoon, Carmen, Agusan del Norte, Philippines

C. spongiosum was wholly dried, coarsely processed in an electric grinder, and weighed 63.0 g. The CUP Series Ethanol Alcohol Extraction System (patented, certified UL 1389 ISO 9001, and PSI) was used to prepare finely powdered material for ethanolic extraction according to the guidelines of Guevara et al. (2005) in DOST Caraga RSTL.

Proximate Analysis

The nutrient composition of raw *C. spongiosum* was analyzed using the Association of Official Analytical Chemists (AOAC) method (2005) at UP Los Banos, BIOTECH, using an oven, muffle (hobersal) furnace, Soxhlet extractor, Kjeldahl apparatus, refluxing apparatus, and etch. The test was to determine the proximate composition of the following: moisture using AOAC 925.45 B, ash using AOAC 923.03, crude fat using AOAC 2003.05, crude fiber using AOAC 978.10, crude protein using AOAC 981.10 (modified) 20th ed., and total sugar using the Phenol Sulfuric Acid Method (Dubois et al. 1956).

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The ethanolic extract of *C. spongiosum* was used to determine the presence of characteristic peaks and their functional groups using FTIR spectroscopy (Perkin Elmer- Attenuated Total Reflectance (ATR)) at the Caraga State University (CSU), Chemistry Laboratory.

Antibacterial Assay

The disk diffusion method (Guevara et al. 2005) was used for the antibacterial assay in DOST Caraga RSTL. Approximately 15 mL of sterile Muller-Hinton's agar was poured into sterile Petri dishes and reconstituted bacterial culture, with adjusted $1.5x10^8$ CFU/mL concentration. Standardized saline suspensions of bacteria were inoculated using sterile cotton swabs into Petri dishes, dried for 15 min, and punched with sterile 6 mm paper discs impregnated with the crude extracts (10 µL). The plates were allowed to stand under aerobic conditions at 37°C for 24 h. The experiment was performed in triplicate. The diameter of the zone of inhibition (ZOI) was evaluated using a Vernier scale millimeter after incubation.

Cytotoxicity Test (Brine Shrimp Lethality Test)

The BSLT assay followed the method described by Guevara et al. (2005) and was performed in the CSU Biology Laboratory. The hatching of Artemia salina Leach eggs was processed using prepared culture media: artificial seawater with 72 g of rock salt in 2 L of distilled water, mild aeration, and under constant illumination (light intensity: 3 watts).

The experimental procedure was performed with five dose treatment groups and three replicates per dose. Each dose consisted of a control group (positive control: potassium dichromate; negative control: 4.5 mL seawater), dose group 1 (100 ppm), dose 2 (10 ppm), and dose 3 (1 ppm).

In 0.5 mL of 80% ethanol (10000 ppm), 0.071 g evaporated plant extract was dissolved. Each concentration (0.5 mL) was diluted to 4.5 mL of 80% ethanol until 100, 10, and 1 ppm (doses) were obtained. Three replicates were performed for each dose. Each replicate contained 4.5 mL of seawater and 0.5 mL of each dose. Ten Artemia salina leach larvae were inserted afterward. A magnifying lens was used to observe and count the number of survivors and larvae deaths (% mortality) after 6 h and 24 h. The LC_{50} value was determined using probit analysis (Finney 1952).

Statistical Analysis

Variations in the bioactivity of the extract on the test bacteria, proximate analysis, and water parameters were analyzed using descriptive statistics in IBM SPSS Statistical Analysis Software. Values were reported as mean±SD. The probit analysis (Finney 1952) was calculated through statistical data with Microsoft Excel to determine LC_{50} in BSLT.

3 Results and Discussion

Classification and Identification of Species

The collected species belong to the class *Florideophyceae* and family *Lomentariaceae*, characterized by creeping axes with erect branches forming turf-like small patches. They grow on rocks or epiphytic, in lower intertidal to upper subtidal (Titlyanov et al. 2017).

Ceratodictyon spongiosum was identified by its form and structure (Figure 2). It was structurally branched with small holes along the branches. It appears sponge-like algae with green-red-purple

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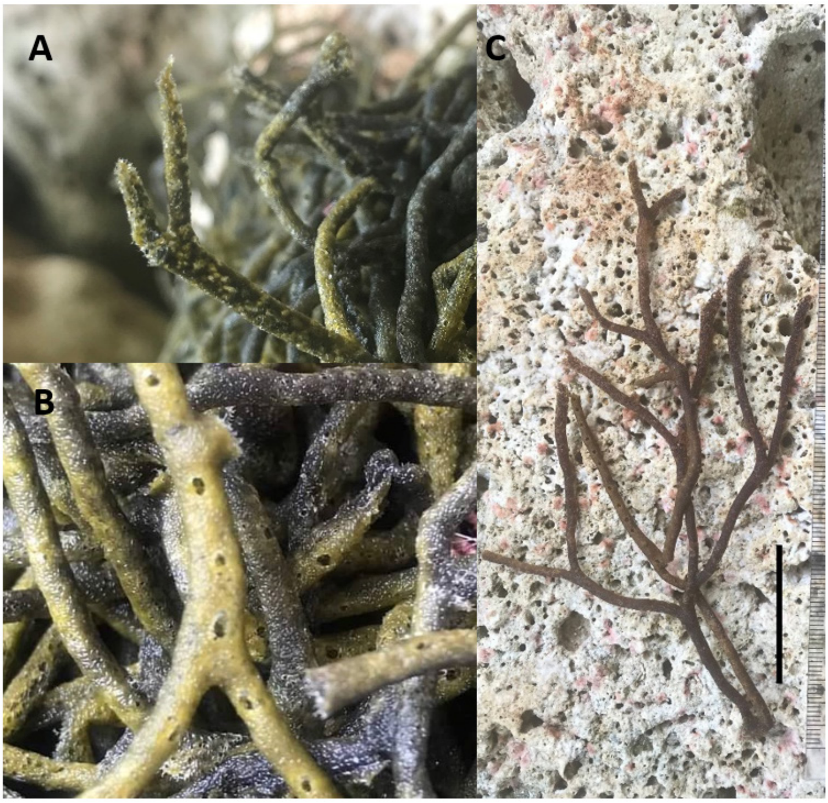

Figure 2. *Ceratodictyon spongiosum* with (A) v-shaped tip, (B) green-red-purple color and branch holes and (C) structural branching. Scale Bar=3cm

in color and a v-shaped tip on its branches. The species measured 3 cm high and 1.0–1.5 cm broad.

Sampling Conditions

Goso-on, Vinapor, Carmen, Agusan del Norte showed standard parameters ideal for the growth and nutritional composition of *Ceratodictyon spongiosum.* The seawater temperature was 34.6°C, with a pH of 7.6, a salinity of 38.3 ppt, a conductivity of 7.6 mS/cm, and an oxidation-reduction potential of 69.5 mV.

Predicting the water quality parameters is a crucial factor in the water quality management of marine waters, which impacts the environment and the species living in it (Ranković et al. 2010). Red algae (*Ceratodictyon spongiosum* Zanardini) in Goso-on, Vinapor, Carmen, Agusan del Norte are ideally related to the obtained water quality parameters that caused the species' inhabitation. It is well known that differences in physical and biological factors may influence seaweed populations' spatial and temporal variations (Schiel & Foster 2006; Wichachucherd et al. 2010). The ideal water temperature for seaweed is between 29°C and 34°C in the wet and dry seasons. Along with the temperature, the pH range (6.5-8.5) (Hidayat et al. 2015) also affects the red seaweed's *C. spongiosum* nutrient composition. Furthermore, Rhodophyta can grow in salinity ranging from 32 to 37 ppt, influencing seaweed reproduction (Ding et al. 2013). Conductivity (5 mS/cm) values depend significantly on temperature and salinity (Ding et al. 2013). The ORP (500–650 mV) is influenced by the water's dissolved oxygen content, which is measured to understand water sanitation levels (Suslow 2004).

Proximate Composition of C. spongiosum

Proximate composition of *Ceratodictyon spongiosum* was expressed as a mean value±SD (Table 1). The moisture content of *C. spongiosum* was 10.28%. The percentage of ash content in *C. spongiosum* was 41.09%, which showed a higher value than other reported Rhodophyta species,

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Table 1. Proximate composition (%) of *Ceratodictyon spongiosum*.

Proximate Composition	Mean ± SD (%)
Moisture	10.28 ± 0.04
Ash	41.09 ± 0.09
Crude Fiber	1.21 ± 0.02
Crude Protein	36.00 ± 0.38
Crude Fat	2.63 ± 0.01
Total Sugar	0.65 ± 0.02

i.e., *Hypnea japonica* (22.10%) (Siddique 2013). Crude protein in *C. spongiosum* displayed a high content of 36%, along with other researched red seaweed, dulse, *Palmaria palmata* (35%), nori algae, *Porphyra umbilicalis* (40%) (Mouritsen et al. 2013; Gamero-Vega et al. 2020). Meanwhile, the crude fat content of the studied red algae was considerably high, at 2.63% relative to the available literature reported by Wong et al. (2000), i.e., *Porphyra umbilicalis* (3.4%). However, the crude fiber of 1.21% and total sugar content of 0.65% of *C. spongiosum* were obtained with a minimal amount compared to the other reported crude fiber of *Tricleocarpa fragilis* (12.05%) and total sugar (24.05%) (Banu & Mishra 2018).

Typically, red algae provide higher-than-average amounts of these constituents (Fleurence et al. 2012; Pereira et al. 2012). Moisture content is an essential standard for identifying the quality of processed seaweed meals, which *C. spongiosum* considers to be quality seaweed. In general, increased ash levels, in turn, indicated a higher mineral content (Gamero-Vega et al. 2020), which was also influenced by the average water salinity relative to the studied results. Moreover, proteins have crucial functions in all biological processes. Because of its excellent nutritional profile, *C. spongiosum's* high content value (36%) would benefit aquaculture and animals as supplemental food (Becker 2004). High-level crude fat contains quality fatty acids such as unsaturated fatty acids (UFA) (Pereira et al. 2012). Subsequently, high amounts of crude fiber are associated with increased satiety, easier digestion, and decreased glucose levels (Brownlee et al. 2005; Mohammed et al. 2021); however, *C. spongiosum* exhibited a low amount of fiber, proving a lack of digestion aid. Total sugar is regarded as the most significant component of metabolism because it provides the essential energy for respiration and other metabolic activities (Alwaleed 2019); otherwise, there is a low amount of total sugar in *C. spongiosum*. Generally, these content values vary by cultivation environment, location, and the sample's age (Sánchez et al. 2007; Siddique 2013).

Ceratodictyon spongiosum, an understudied red alga, was strongly suggested in this study for its promising proximate composition (%). Compared to other species of seaweed, red seaweed, or Rhodophyta, is thought to be a considerably more significant source of biologically active metabolites (Mouritsen et al. 2013; Shannon & Abu-Ghannam 2019; Naseri et al. 2019; Gamero-Vega et al. 2020). This emergence is subject to novel products in the health food industry, as it is one of the primary producers of organisms in various habitats (Saranraj 2014).

FTIR Spectra of C. spongiosum

The obtained analysis showed the IR spectrum of the C. spongiosum extract peaking at wavenumbers 3339, 2997, 2910, 1640, 1453, 1043, and 880 cm^{-1}, which exhibited seventeen functional groups (Table 2). The sample's strong bands at around 3339 cm^{-1} may be attributed to the O–H and N–H stretching, indicative of amino acids (Kannan 2014; Anjali et al. 2019). Kalasariya (2020) stated that peaks at 2997 cm^{-1} are formed due to the O-H stretch of alcohol and the C-H stretch of alkene. The N-H stretch of amine salt, the O-H stretch of carboxylic acid, and the C-H stretch of alkane can be found at the peak of 2910 cm^{-1}. The peak at 1640 cm^{-1} is attributed to the N-H bending of amine, representing the C=C stretch of alkene, conjugated alkene, and cyclic alkene. It is also owed to the C=O, which indicates the presence of the ester group. Additionally, peak 1453 cm^{-1} is formed due to the C-H stretch of alkane. Peak 1043 cm^{-1} indicates the C-N stretch of primary amine, and peak 880 cm^{-1} represents the C-H bend and C-O-O stretch of the aromatic ring and ether oxy, respectively.

Table 2. FTIR peak values and functional group for sample *Ceratodictyon spongiosum*

Absorption peak (cm^{-1})	Peak appearance	Functional Group	Class	References
3339	Strong, Broad Strong, Broad	O-H Stretching N-H stretching	Alcohol Amino acid	Kannan (2014); Anjali et al. (2019)
2977	Strong, Broad Medium	O-H Stretching C-H Stretching	Alcohol Alkane	
2910	Strong Strong Medium	N-H Stretching O-H Stretching C-H Stretching	Amine salt Carboxylic acid Alkane	Kalasariya (2020)
1640	Medium Medium Medium Medium Strong	N-H bending C=C Stretching C=C Stretching C=C Stretching C=C Stretching C=O stretching	Amine Alkene Conjugated alkene Cyclic alkene Alkene Ester	
1453	Medium	C-H stretching	Alkane	
1043	Strong	C-N stretch	Primary amine	Nandiyanto et al. (2019)
880	Medium	C-H bending C-O-O stretch	Aromatic ring Ether, oxy	

The functional group, distinguished by FTIR spectrum analysis, might confirm the potential antioxidant activity to fight against various diseases and free radical scavenging properties—the Gracilaria dura (Kalasariya 2020) correlated with the functional group present in *C. spongiosum.*

Antibacterial Activity of C. spongiosum

The diameter of antibacterial for each triplicate in different bacteria showed a relatively high percentage of inhibition (Table 3). The *C. spongiosum* extract against *S. aureus* displayed a 24.67±0.58 mm zone of inhibition (ZOI) and against *E. coli* with 22.67±0.58 mm ZOI. The extract showed high-level susceptibility to antibacterial activity. A diameter <10 mm is expressed as inactive, 10-13 is partially active, 14-19 is active, and a diameter >19 mm exhibits a very functional outcome for the zone of inhibition (Guevara et al. 2005).

It was indicated (Table 3) that gram-positive *S. aureus* provides the highest zone of inhibition. Suggesting that ethanol extracts of red alga *C. spongiosum* are more efficiently bioactive against Gram-positive bacteria. Similar to a study presented by Kolanjinathan et al. (2009) and Karabay-Yavasoglu et al. (2007).

This study also suggests that 80% ethanol is a better solvent for consistently extracting bioactive compounds from red seaweeds. It might be due to the capacity of ethanol to elute compounds responsible for their activity. However, the efficacy of ethanol depends on the biochemical composition of red algae. The bioassay methods, geographical distribution of seaweeds, and seasonal production of bioactive compounds also contribute to the efficient bioactive properties of seaweeds (Salvador et al. 2007).

Cytotoxic Activity

The study determined the extent of lethality corresponding to the concentration of the *Ceratodictyon spongiosum* ethanolic extract (Table 4). After 6 h of observation, all shrimps survived in negative and positive control from concentrations 100, 10, and 1 ppm (ug/mL). The highest mortality in *A. salina* was observed in 100 ppm negative and positive control after 24 h. At 6 h, the extract of *C. spongiosum* caused the lowest mortality in all concentrations; 1 ppm, 10 ppm, and 100 ppm with 0%, 1.11%, and 3.33% mortality, respectively. However, at 24 h, extracts caused average mortality in 1 ppm and 10 ppm with 45.56% and 96.67% mortality, respectively, and a higher lethality percentage in 100 ppm with 100% mortality. The 24 h assay showed more significant mortality than 6 h.

The LC_{50} value obtained after 6 h of exposure was 928.86 ppm, which displayed the sample extract as slightly toxic to nauplii. An LC_{50} value obtained after 24 hours was 0.96 ppm which is highly toxic to nauplii. An extract is considered highly toxic if the LC_{50} value is <100 ppm, moderately toxic when the LC_{50} value is 101-500 ppm, slightly toxic when the value is 501-1000 ppm, and non-toxic when LC_{50}> 1000 ppm. The LC_{50} is inversely proportional to toxicity (Meyer et al. 1982; Clarkson et al. 2004).

Table 3. Zone of inhibition (mm) of seaweed extract against *E. coli* and *S. aureus*

Treatment	Bacteria	(mean ± SD)	Remarks
Extract *Ceratodictyon spongiosum*	*Escherichia coli*	22.67 ± 0.58	Very active
	Staphylococcus aureus	24.67 ± 0.58	Very active

Table 4. Cytotoxic activity of Ethanolic extract *C. spongiosum* evaluated after 6 and 24 hours.

Hours	Concentration (mg/L/ppm)	No. of Survivor			% Mortality	LC50 (ug/mL)	Toxicity Criterion
		R1	R2	R3			
6 h	1	30	30	30	0.00	928.86	Slightly toxic
	10	30	29	30	1.11		
	100	29	29	29	3.33		
24 h	1	0	0	0	45.56	0.96	Highly toxic
	10	0	2	1	96.67		
	100	22	19	8	100.00		

The findings showed that *C. spongiosum* contains cytotoxic substances which could be explored further to be used therapeutically. Manilal et al. (2009) suggested that secondary metabolites extracted and the polarity of the different compounds may influence the cytotoxic activity of the red seaweed species. Alencar et al. (2014) also showed a lethality test of red seaweeds, which suggested that lethality against *Artemia* sp. was dose-dependent.

4 Conclusions and Recommendations

Typically, red seaweeds provide higher-than-average amounts of biochemical compositions than brown and green seaweeds. The findings of our study demonstrate that water parameters significantly impact the biochemical makeup of red seaweed. It showed that the amounts of protein, ash, moisture, crude fat, crude fiber, and total sugar in *Ceratodictyon spongiosum* were high to average. These could be used as food and feed resources for human and animal consumption, with high-quality nutritional content. Additionally, these seaweeds could be rich sources of natural antioxidants because of the functional group's presence. Findings on the antibacterial and cytotoxic activity can be a basis for more advanced research on red seaweed's capabilities, enriching the national pharmaceutical industry for nutritional, medical, and industrial aspects.

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5 Statement of Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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